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A Single Mutational Modification of a Tryptophan-specific Transfer RNA Permits Aminoacylation by Glutamine and Translation of the Codon UAG

MOSHE YANIV†, WILLIAM R. FOLK‡, PAUL BERG

Department of Biochemistry
Stanford University Medical Center
Stanford, Calif., U.S.A.

AND

LARRY SOLL§

Department of Microbiology and Molecular Genetics
Harvard Medical School
Boston, Mass., U.S.A.

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In this work we show that the wild-type (su^-7) progenitor of the recessive-lethal suppressors of UAG (su^+7(UAG)) and of UAA/G (su^+7(UAA/G)) is the structural gene for transfer RNA^{Trp}, the adaptor for translating the codon UGG. The su^+7(UAG) suppressor form of the tRNA has a C for U substitution in the middle base of the anticodon; in the su^+7(UAA/G) suppressor tRNA both C residues of the anticodon are replaced by U. Our data establish that the mutational change altering the $tRNA^{Trp}$ to a UAG suppressor is accompanied by a loss of tryptophan-accepting specificity and the acquisition of glutamine-acceptor activity.

1. Introduction

Much of the insight for our recent progress in understanding the structure–function relations of transfer RNA has come from studies of mutational changes that affect specific tRNA molecules (see for example Berg, 1972; Smith, 1972). Such mutational alterations have clarified certain aspects of tRNA synthesis and processing (Altman & Smith, 1971), translation (Carbon *et al.*, 1970; Riddle & Roth, 1970; Riddle & Carbon, 1973), aminoacylation (Carbon & Curry, 1968; Squires & Carbon, 1971; Abelson *et al.*, 1970) and cellular regulation (Singer *et al.*, 1972).

One of the difficulties of this approach is that the mutational alteration may inactivate an indispensible tRNA function and thereby be lethal. Several years ago, Soll & Berg (1969*a*) discovered such a class of UAG and UAA suppressors; similar findings were made by Miller & Roth (1971) for a UGA suppressor. Soll &

† Present address: Department of Molecular Biology, Pasteur Institut, 25 rue du Docteur Roux, Paris 15eme, France.

‡ Present address: Department of Biological Chemistry, University of Michigan School of Medicine, Ann Arbor, Mich., U.S.A.

§ Present address: Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colo., U.S.A.

Berg (1969*b*) established that their UAG suppressor, *su*$^+$*7*(UAG)†, caused the insertion of glutamine at positions specified by UAG, and preliminary unpublished observations (Cordes, Primakoff & Berg) indicated that tRNA isolated from *su*$^+$*7*(UAG), but not *su*$^-$*7*, *Escherichia coli* strains, suppressed the chain termination effect of UAG *in vitro*. From this we inferred that the mutationally altered tRNA was most likely a $tRNA^{Gln}$ species which could translate UAG. As a first step to proving this the nucleotide sequences of the two wild-type $tRNA^{Gln}$ species were determined (Folk & Yaniv, 1972) but because of difficulties in the purification of the *su*$^+$*7*(UAG) suppressor tRNA we were unable to identify its origin.

The availability of the phage ϕ80d*su*$^+$*7*(UAG) and its lysogens (Soll, 1974) made it possible to increase the intracellular concentration of the *su*$^+$*7*(UAG) tRNA and to purify it sufficiently to identify the wild-type tRNA from which it is derived. Unexpectedly, the *su*$^+$*7*(UAG) tRNA, which inserts glutamine in response to UAG, is derived from $tRNA^{Trp}$, which normally translates UGG as tryptophan. Thus, the substitution of a C for a U in the anticodon alters both the coding specificity and amino acid-acceptor specificity. Since the tRNA needed to translate UGG as tryptophan is unique (Hirsh, 1971) and is lost by the *su*$^+$*7*(UAG) mutation, it is not surprising that the mutation is lethal in the haploid state (*su*$^+$*7*) but is innocuous in heterozygous cells (*su*$^+$*7*/*su*$^-$*7*).

2. Materials and Methods

(a) *Reagents and enzymes*

Unlabeled L-amino acids, ATP and crystalline bovine serum albumin were A grade products purchased from Calbiochem. Phenoxyacetyl-*N*-hydroxysuccinimide was a gift from Dr A. M. Michelson. Crystallized acrylamide, bisacrylamide and tetramethylene diamine were purchased from Eastman. Yeast RNA for homochromatography was a British Drug Houses product. [^{14}C]glutamine, [^{14}C]valine and carrier-free [^{32}P]phosphoric acid were purchased from New England Nuclear. [^{3}H]tryptophan (a gift from R. Denney) was purchased from Schwarz and purified before use on Dowex-1 (8% cross-linked). Benzoylated DEAE-cellulose was either purchased from Schwarz (lot 6902) or prepared according to Gillam *et al.* (1967). DEAE-cellulose DE52 powder and DE81 chromatography paper were Whatman products and DEAE-Sephadex A50 was from Pharmacia. Cellulose acetate strips were purchased from Schleicher & Schüell. Cellulose powder MN300H and DEAE cellulose powder MN300G for thin-layer chromatography were Macherey & Nagel products. Kodak RP/S X-ray film was used for autoradiography.

Ribopolymers poly(A, U) (1:1) and poly(A, C) (1:1) and poly(U,A,G) (1:1:1) were purchased from Miles Laboratories: the triplets CAG and UAG were prepared by incubation of the respective dinucleotides (from Miles) with GDP in the presence of polynucleotide phosphorylase (a gift from Dr M. Singer) and ribonuclease T_1 (Sundarajan & Thach, 1966); the triplets obtained were dephosphorylated with *E. coli* alkaline phosphatase.

Ribonucleases T_1, T_2 and U_2 were Sankyo products. *E. coli* alkaline phosphatase and pancreatic ribonuclease (RAF) were purchased from Worthington. Glutaminyl-tRNA synthetase was purified as described by Folk (1971); valyl-tRNA synthetase was purified

† Abbreviations used: *su*$^-$*7* refers to the wild-type, non-suppressing allele. *su*$^+$*7* refers to one of the mutationally altered suppressing forms: *su*$^+$*7*(UAG), the amber UAG suppressor; *su*$^+$*7*(UAA/G), the ochre or UAA/G suppressor; *su*$^+$*7*(UGA-1) is the UGA suppressor which has a base change at position 24 of $tRNA^{Trp}$ (Hirsh, 1971) and *su*$^+$*7*(UGA-2) is the UGA suppressor presumed to have a base change in the anticodon of the $tRNA^{Trp}$ (Soll, 1974). The suppressed codons are not indicated in parentheses where the intent is not relevant to the statement. Aminoacyl-tRNA synthetases are abbreviated by using the standard three-letter designation for each amino acid followed by the letters RS, e.g. ValRS, GlnRS etc.

TABLE 1

Bacterial strains

Strain	Genotype	Prophage
LS446	*pro*, *his29*(UAG), *trpA9605*, *su*$^+$*7*(UAG), *ilv*$^-$/*su*$^-$*7*, *ilv*$^+$	
LS633	*lacZy14*(UAG), *trpA9605*, *mal 8*(UAG), *ilvD493*	(ϕ80h) (ϕ80d$_2$*ilv*$^+$*su*$^+$*7*(UAG))
LS649	*lacZy14*(UAG), *trpA9605*, *mal 8*(UAG), *ilvD493*	(ϕ80h) (ϕ80d$_2$*ilv*$^+$*su*$^-$*7*)
LS650	*lacZy14*(UAG), *trpA9605*, *mal 8*(UAG), *ilvD493*	(ϕ80h) (ϕ80d$_8$*ilv*$^+$*su*$^-$*7*)
MY202	*argA*, *trpA9605*(UAG), *leu*(UAG), *rel*	(ϕ80h) (ϕ80d$_1$*su*$^+$*7*(UAG))
PP113	*lacZ*(UAG), *argA*, *glyA34*, *trpA36*, *strA*	
LS639	*trpR*, *trpE9851*(UAA), *his29*(UAG), *ilv1*	(ϕ80h) (ϕ80d$_1$*su*$^+$*7*(UAA/G))

The isolation of the first four bacterial strains is described in the preceding paper (Soll, 1974). Strain MY202 was derived from BF266 (Folk & Berg, 1970) by lysogenization with ϕ80, and strain PP113 (Primakoff & Berg (1970)) from BF265 (Folk & Berg, 1970).

as described by Yaniv & Gros (1969). A fraction containing all the aminoacyl-tRNA synthetases was prepared as described by Muench & Berg (1966) and was a gift from P. Primakoff. Partially purified *E. coli* $tRNA^{Trp}$ was a gift of C. Squires. *E. coli* 70 S ribosomes were prepared according to Nirenberg & Leder (1964).

(b) *Strains*

The genotypes of all of the bacterial strains used in this study are described in Table 1.

(c) *Preparation of phage stocks*

Phage infection or induction of lysogenized bacteria was performed as described by Soll (1974). The phage was concentrated by centrifugation for 10 h at 8000 *g* and resuspended in a small volume of 50 mM-Tris·HCl (pH 7·3), 20 mM-$MgSO_4$.

(d) *Labeling of tRNA made after infection*

Strain PP113 was grown overnight in low-phosphate medium (M70) containing 100 mM-Tris·HCl (pH 7·5), 20 mM-$MgSO_4$, 3 mM-KCl, 0·01 mM-$FeCl_3$ supplemented with 1 mM-potassium phosphate, 0·4% glucose, 100 μg arginine/ml, 50 μg glycine/ml and 25 μg tryptophan/ml. The overnight culture was diluted 1:75 with fresh medium containing 1 mM-phosphate. Cells were harvested at an absorbance of 0·5 (A_{590}), centrifuged at 20°C for 10 min at 8000 *g* and resuspended at an A_{590} of 5 units in 10 mM-Tris·HCl (pH 7·3), 15 mM-$MgSO_4$ and 1 mM-$CaCl_2$ at 37°C. Phage were added at a multiplicity of 10 infective particles (containing about 15% defective transducing particles) per cell, and after absorption for 20 min at 42°C, the infected cells were diluted tenfold in fresh M70 medium containing 0·1 mM-phosphate and 0·2 mCi [^{32}P]phosphate/ml. After 45 min of vigorous aeration at 37°C, chloramphenicol (80 μg/ml) was added and the cells were aerated for another 3 h.

(e) *tRNA extraction*

^{32}P-labeled tRNA from phage-infected cells was isolated as follows: 10 ml of a ^{32}P-labeled culture, described above, was centrifuged, the cells were washed with 0·9% KCl and resuspended in 1 ml of a solution containing 20 mM-Tris·HCl (pH 7·4), 20 mM-NH_4Cl, 5 mM-magnesium sulphate and 0·1 mM-EDTA. Water-saturated phenol was added (1 ml) and the tRNA was extracted by shaking at room temperature for 30 min. After centrifugation the supernatant was collected, non-radioactive carrier tRNA (160 μg) was added and the tRNA was precipitated by addition of 0·1 vol. 2 M-potassium acetate (pH 5·2) and 2 vol. ethanol. The pellet obtained by centrifugation was dissolved in 0·5 ml of 0·1 M-Tris·HCl (pH 9·1) and incubated for 30 min at 30°C. The tRNA was then reprecipitated with ethanol, centrifuged and dissolved in 0·3 ml of 0·2 M-potassium acetate (pH 5·0). After another ethanol precipitation the pellet was washed several times with 1 ml of 75% aqueous ethanol and finally dissolved in 40 μl water, and centrifuged to remove any insoluble ^{32}P-labeled ribosomal RNA. Non-radioactive tRNA was prepared as described by Richardson (1966).

(f) *Polyacrylamide gel electrophoresis*

The general conditions for the 40-cm slab polyacrylamide gel electrophoresis, described by Adams *et al.* (1969), were followed. The acrylamide concentration was 12·5% with 0·25% bisacrylamide in 0·1 M-Tris acetate (pH 8·3), 0·1 mM-EDTA. Electrophoresis was performed for 16 h at 400 V and then the gels were autoradiographed and the tRNA bands eluted by homogenization with 2 ml of 0·1 M-Tris·HCl (pH 9·1), 0·5 M-NaCl, 10 mM-EDTA and carrier tRNA (65 μg). After 30 min incubation at 37°C the suspension was filtered through glass wool, the pellet was washed with 1 ml of the elution solution (without carrier) and the filtrate was precipitated with ethanol. The precipitate was collected by centrifugation, washed successively with 67% aqueous ethanol in 0·5 M-NaCl, 75% aqueous ethanol and dissolved in a small volume of water.

(g) *Sequencing techniques*

The methods developed by Sanger and his collaborators for sequencing [^{32}P]RNA were followed throughout this work (Sanger *et al.*, 1965; Brownlee & Sanger, 1967,1969;

Brownlee *et al.*, 1968). Electrophoresis buffers contained 10^{-4} M-EDTA to improve the sharpness of spots. Ribonuclease U_2 digestion of oligonucleotides, isolated after homochromatography, was for 2·5 h at 37°C with 1 unit of enzyme/ml containing 0·1 mg bovine serum albumin/ml, 50 mM-sodium acetate (pH 4·5) and 2 mM-EDTA. The RNAase U_2 digestion products were separated on DEAE-cellulose paper in 7% formic acid. Reaction of oligonucleotides with the water-soluble carbodiimide *N*-cyclohexyl-*N'*(β-morpholinyl-(4)-ethyl)carbodiimide methyl-*p*-toluene sulfonate, and subsequent digestion with pancreatic RNAase, were carried out according to Cory & Marcker (1970).

(h) *Aminoacylation of tRNA*

Assays for the acceptor capacity of tRNA fractions were done as follows: charging of valine was done with purified ValRS (Yaniv & Gros, 1969), of glutamine with purified GlnRS (Folk, 1971) and of tryptophan with an S-100 protein fraction (Muench & Berg, 1966). Large amounts of [^{14}C]glutaminyl-tRNA were prepared by increasing the size of the reaction mixture used for assaying tRNA^{Gln}. The aminoacylated tRNA was isolated as described by Muench & Berg (1966). Phenoxyacetylation of glutaminyl-tRNA was done as described by Gillam *et al.* (1968).

3. Results

(a) *Does* su$^+$7 *code for a UAG-specific transfer RNA*^{Gln} *species?*

The amber suppressor, *su*$^+$*7*, promotes the insertion of glutamine into protein in response to the UAG codon (Soll & Berg, 1969*b*). Preliminary experiments *in vitro* involving translation of a phage RNA message containing an amber codon indicated that a tRNA, specific to *su*$^+$*7* cells (compared with *su*$^-$*7* cells), was responsible for the suppression (Cordes, Primakoff & Berg, unpublished observations). Glutaminyl-tRNA from a *su*$^+$*7*/*su*$^-$*7* diploid strain (Soll & Berg, 1969*a*) (prepared by aminoacylating either bulk tRNA or partially purified tRNA^{Gln}) could also bind to ribosomes with the triplet UAG (our unpublished results); but this occurred only if the glutaminyl-tRNA had been made using a large excess (100-fold) of glutaminyl-tRNA synthetase. With lower quantities of GlnRS (30-fold less) the quantity of glutaminyl-tRNA formed was not markedly reduced, but very little of the glutaminyl-tRNA was bound to UAG–ribosome complexes. Of importance was the fact that glutaminyl-tRNA binding to UAG was detected only with tRNA from cells carrying the *su*$^+$*7*(UAG) allele. This suggested that the UAG-specific suppressor tRNA occurred only in *su*$^+$*7*(UAG) cells and was poorly charged with glutamine.

Support for this suggestion was obtained when chromatography of the mixed tRNA from strain LS446 on DEAE-Sephadex (Nishimura *et al.*, 1967) revealed three separated peaks of glutamine acceptor when the fractions were assayed with high levels of GlnRS (open bars) but only two (peaks 2 and 3) when the assays were done with low levels of GlnRS (solid bars) (Fig. 1). Samples of each of the three peaks were pooled, concentrated and aminoacylated with [^{14}C]glutamine using a high concentration of GlnRS; each fraction was then tested for ribosome–codon binding, as described by Nirenberg & Leder (1964). The results (Table 2) show that the RNA in the first peak (which is inefficiently aminoacylated by GlnRS) binds specifically to UAG whereas the tRNAs in the second and third peaks are specific for the known glutamine codons, CAA (poly(A,C)) and CAG, respectively. As expected, only the latter two peaks of tRNA^{Gln} were found in tRNA from *su*$^-$*7* cells. The nucleotide sequences of tRNA$_1^{Gln}$ (peak 2) tRNA$_2^{Gln}$ (peak 3) have already been established (Folk & Yaniv, 1972): tRNA$_1^{Gln}$ has an anticodon sequence (5′) NUG where N is probably

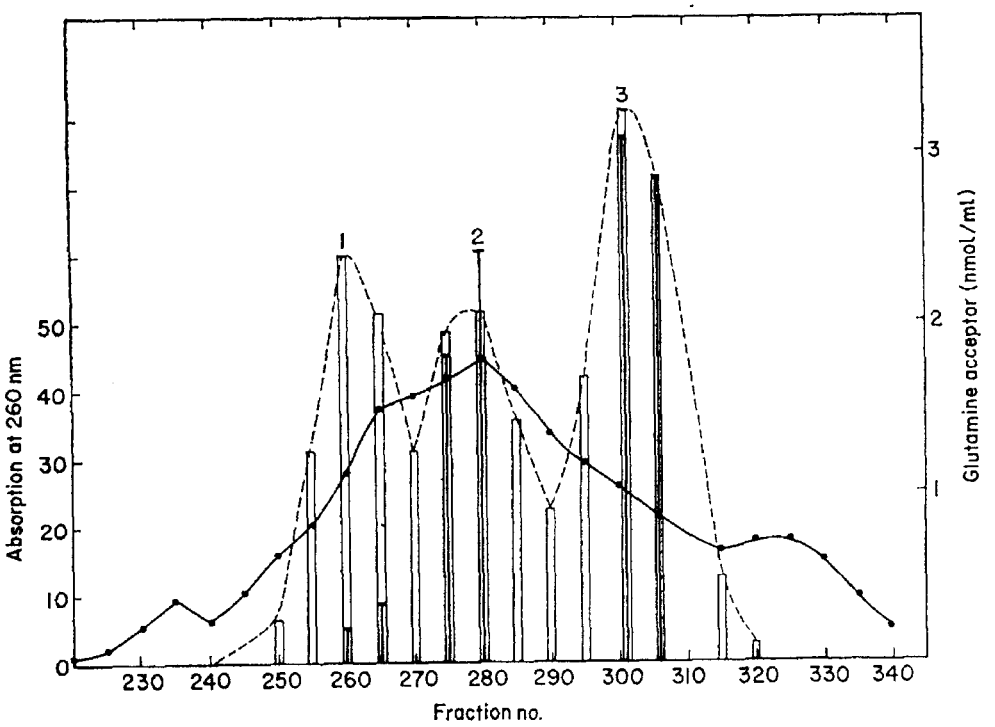

FIG. 1. DEAE-Sephadex chromatography of tRNA from an *su*$^+$*7*(UAG)/*su*$^-$*7* diploid strain. An *su*$^+$*7*(UAG)/*su*$^-$*7* diploid strain (LS446) was inoculated into a supplemented minimal medium, the overnight culture was diluted in twofold concentrated broth (20 g Tryptone, 10 g yeast extract, 10 g NaCl, per liter) and the culture grown to late exponential phase. Total tRNA was extracted as described in Materials and Methods. The tRNA (3·6 g) was fractionated on a DEAE-Sephadex column, the different acceptor activities were partially separated by elution with a gradient formed between 0·375 M-NaCl, 20 mM-Tris·HCl (pH 7·5), 9 mM-$MgCl_2$, 0·1 mM-EDTA and 0·525 M-NaCl, 20 mM-Tris·HCl, 16 mM-$MgCl_2$, 0·1 mM-EDTA. Acceptor activity for glutamine was assayed on 5-μl portions with 1 μg of purified GlnRS (high enzyme) or 0·03 μg of GlnRS (low enzyme) in a volume of 0·1 ml using the conditions described by Folk (1971). —●—●—, Absorption at 260 nm. The open vertical bars are the glutamine acceptor values with the high level of GlnRS and the heavy vertical bars are the glutamine acceptor values with ow levels of GlnRS.

TABLE 2

Coding properties of separated transfer RNA^{Gln} species

Polymer or triplet	Peak 1 $tRNA^{Gln}_{su+7}$	Peak 2 $tRNA_1^{Gln}$	Peak 3 $tRNA_2^{Gln}$
poly(U,A)	0·07	0·06	0·07
poly(A,C)	0·11	0·51	0·19
poly(U,A,G)	0·23	0·1	0·1
CAG	0·09	0·12	0·8
UAG	0·6	0·06	0·11

tRNA samples from peaks 1 to 3 of the DEAE-Sephadex column eluate: fractions 260 (*su*$^+$*7*-(UAG) $tRNA^{Gln}$), 280 ($tRNA_1^{Gln}$) and 301 ($tRNA_2^{Gln}$) were concentrated and aminoacylated using [^{14}C]glutamine and a high concentration of GlnRS. Approximately 10 pmol [^{14}C]glutaminyl-tRNA were assayed in the ribosome binding assay by the procedure of Nirenberg & Leder (1964) in a volume of 50 μl containing 50 mM-Tris·HCl (pH 7·0), 50 mM-potassium acetate, 20 mM-magnesium acetate and 0·1 mM-EDTA, 1 A_{260} unit of ribosomes and 0·27 A_{260} unit of trinucleotides. Values given are in pmol bound to the nitrocellulose filters.

a 2-thiouridine derivative that will not hydrogen bond with G (Yoshida *et al.*, 1970), consequently it is specific for CAA; $tRNA_2^{Gln}$ has the anticodon sequence CUG, which should pair only with CAG. Thus, the specificities observed in the triplet binding assay (Table 2) agree with those predicted from the sequence data (Folk & Yaniv, 1972).

Attempts were made to purify the UAG-specific tRNA (peak 1) from the *su*$^+$*7*/*su*$^-$*7* heterozygote by methods that proved successful for $tRNA_1^{Gln}$ and $tRNA_2^{Gln}$: DEAE-Sephadex column chromatography followed by benzoylated DEAE-cellulose fractionation, acylation with a large excess of GlnRS, then phenoxyacetylation (Gillam *et al.*, 1968) and rechromatography on benzoylated DEAE-cellulose. However, at each step there were particularly high losses of that specific glutamine acceptor activity and it was not possible to carry out a nucleotide sequence analysis of that tRNA species.

(b) *Multiplication of φ80d*su$^+$*7(UAG) DNA causes an increase in the suppressor transfer RNA*

Several groups (Primakoff & Berg, 1970; Squires & Carbon, 1971) have made use of the discovery by Smith *et al.* (1966) that intracellular multiplication of a phage carrying the structural genes for tRNAs causes a striking increase in the level of those tRNAs. To determine if the *su*$^+$*7*(UAG) allele is the structural gene for the suppressor tRNA, we examined the consequences of induction (after ultraviolet irradiation) of a *φ*80d*su*$^+$*7*(UAG) lysogen of *E. coli* (actually a double lysogen of *φ*80d*su*$^+$*7*(UAG) and *φ*80). Amino acid-acceptor assays were performed on the bulk tRNA isolated after induction and the values were normalized to the valine-acceptor levels (Table 3). The total $tRNA^{Gln}$ level increased about twofold by four hours after induction. A more meaningful measurement is the increment in the $tRNA^{Gln}$ species, which is aminoacylated only with high levels of GlnRS. With tRNA from uninduced cells, when high levels of GlnRS are used, only five picomoles more of glutamine per A_{260} unit is charged but with tRNA from induced cells the comparable value is 54 picomoles per A_{260}. Normalized to $tRNA^{Val}$ in each case, there is more than a tenfold increase in the suppressor tRNA in cells multiplying *φ*80d*su*$^+$*7*(UAG) phage DNA. This is not a non-specific effect of u.v. induction since essentially similar results were obtained after infection of *E. coli* with a mixture of *φ*80 and *φ*80d*su*$^+$*7*(UAG).

(c) *Lysogens of φ80d*su$^-$*7 have lower transfer* RNA^{Gln} *levels and higher amounts of transfer* RNA^{Trp} *than lysogens of φ80d*su$^+$*7(UAG)*

If the suppressor $tRNA^{Gln}$ is derived from one of the normal $tRNA^{Gln}$ species then replacement of the *su*$^+$*7*(UAG) allele by *su*$^-$*7* should not alter the amount of $tRNA^{Gln}$. On the other hand, if the *su*$^-$*7* allele corresponds to a different tRNA species, we would expect that the amount of that tRNA would be elevated in the *su*$^-$*7* lysogen. Accordingly the levels of several different tRNA acceptors were measured in cells lysogenic for *φ*80d*su*$^+$*7*(UAG) or its revertant *φ*80d*su*$^-$*7* (Table 4). In two different *φ*80d*su*$^-$*7* lysogens, the amount of $tRNA^{Gln}$ (measured with high levels of GlnRS) was about 50 to 70% that of the *φ*80d*su*$^+$*7*(UAG) parent when normalized to $tRNA^{Val}$.

Particularly interesting is the observation that the *φ*80d*su*$^-$*7* lysogen contains

TABLE 3

Increase in transfer RNA^{Gln} following induction of cells doubly lysogenic for $\phi 80 d su^+7(UAG)$ and $\phi 80$ phages

Source of tRNA	$tRNA^{Gln}$ With high levels of GlnRS (H)	$tRNA^{Gln}$ With low levels of GlnRS (L)	$tRNA^{Val}$	Ratio (H) minus (L)/$tRNA^{Val}$
Non-induced	31	26	61	0·08
Induced (4 h)	77	23	68	0·79

A double lysogenic strain MY202 was grown in a supplemented minimal citrate medium (Vogel & Bonner, 1956) and induced by u.v. irradiation at a density of 3×10^8 cells/ml. Cells were diluted with an equal volume of medium and 45 min later 50 μg valine were added/ml to inhibit lysis. Aeration was continued for 4 h, cells were collected and the tRNA isolated as described in Materials and Methods.

H and L refer to the level of $tRNA^{Gln}$ determined with high and low levels of GlnRS, respectively.

TABLE 4

Comparison of transfer RNA^{Gln} and transfer RNA^{Trp} levels in $\phi 80$su$^+$7(UAG) and $\phi 80$su$^-$7 lysogens

Source of tRNA	$tRNA^{Gln}$	$tRNA^{Trp}$ (pmol/A_{260} nm)	$tRNA^{Val}$	Ratio $tRNA^{Gln}/tRNA^{Val}$	Ratio $tRNA^{Trp}/tRNA^{Val}$
LS633 (*su*$^+$7(UAG))	31	12	74	0·42	0·16
LS649 (*su*$^-$7)	18	27	63	0·29	0·43
LS650 (*su*$^-$7)	14	26	73	0·19	0·36

tRNA was prepared from an exponentially growing culture in supplemented minimal citrate medium as described in Materials and Methods. A high level of GlnRS was used to charge the $tRNA^{Gln}$ species.

two- to threefold more $tRNA^{Trp}$ relative to $tRNA^{Val}$ than the $\phi 80 d su^+7$(UAG) lysogen. We tested the $tRNA^{Trp}$ levels because of Miller & Roth's suggestion (1971) that the locus in *Salmonella typhimurium* corresponding to the *su7* locus on the *E. coli* chromosome might also be responsible for the recessive lethal UGA and UAG suppressors in that organism. Soll's observation (Soll, 1974) that the su^-7 allele of $\phi 80 d su^-7$ could be mutationally altered to yield either a UGA or UAG suppressor also reinforced that notion. If that is the case, $tRNA^{Trp}$ is the only tRNA in which single base changes in its anticodon would permit translation of UAG or UGA. Moreover, if such a change occurred, it would very likely be lethal in a strain carrying only a single copy of that gene since it would eliminate the only tRNA capable of translating the tryptophan codon UGG (Hirsh, 1971).

(d) *The nucleotide sequence of the UAG suppressor transfer RNA^{Gln} is that of a modified transfer RNA^{Trp}*

The proof that the su^+7 locus codes for a modified $tRNA^{Trp}$ species was obtained from nucleotide sequence analysis of the tRNAs produced after infection with $\phi 80$ phages carrying different alleles of the *su7* locus. To circumvent the previously described difficulty encountered in purifying the UAG-specific tRNA from su^+(UAG) strains, the bulk tRNA produced after infection of a strain with $\phi 80 d su^+7$(UAG) was fractionated by polyacrylamide slab gel electrophoresis (Adams *et al.*, 1969; Smith *et al.*, 1970). The major tRNA band was divided into slow and fast-migrating fractions, and the tRNA in each was analyzed by two-dimensional electrophoresis after complete RNAase T_1 digestion. In spite of the fact that the digest of the fast-moving fraction yielded a complex pattern of spots on the autoradiogram (Plate I(a)), several of the spots were characteristic of those derived from $tRNA^{Trp}$: spots 5 to 12 and 14, after elution, digestion with pancreatic RNAase or RNAase T_2 and analysis for minor bases, contained oligonucleotides identical to those reported by Hirsh (1971) for $tRNA^{Trp}$. Ordinarily $tRNA^{Trp}$ is present in less than 1% of the total tRNA and its oligonucleotides would not be distinguished in an RNAase T_1 digest of the total RNA. For example, infection with $\phi 80 p su^+3$(UAG) (the phage carrying the su^+3 gene induces the synthesis of a modified $tRNA^{Tyr}$ species (Smith *et al.*, 1966)) generated only traces of the $tRNA^{Trp}$ oligonucleotides by the same procedures.

The sequence of the oligonucleotide in spot 13 (Plate I(a)) resembled that of the oligonucleotide containing the $tRNA^{Trp}$ anticodon. Pancreatic RNAase digestion of this oligonucleotide yielded two fragments diagnostic of the $tRNA^{Trp}$ anticodon: A-$\overset{16}{A}$-A-A-C and C_m-U. The analysis of the sequence of this oligonucleotide is given in Table 5. The location of the isopentenyl group was not fixed among the four adenosine residues (in $tRNA^{Trp}$ the isopentenyl group is on the second adenosine from the 5′ terminus, i.e. the adenosine adjacent to the anticodon triplet). The sequence of this oligonucleotide from su^+7(UAG) tRNA differs from the sequence of the corresponding oligonucleotide in $tRNA^{Trp}$ by a U for C substitution in the second base of the anticodon (see Fig. 2 for summary)†. This change explains how a $tRNA^{Trp}$,

† One minor difference observed between our sequences and the one published by Hirsh (1971) is the absence of a second dihydrouridine residue in the sequence U-U-C-C-A-A-D-U-G (Hirsh reported U-U-C-C-A-A-D-D-G). It is possible that this lack of a dihydrouridine resulted from undermodification of the tRNA after phage infection, as observed with $tRNA^{Tyr}$ (Gefter & Russell, 1969). Frequently, even without infection, a DD sequence is accompanied by a DU

Plates I and II

Plate I. Autoradiogram of a two-dimensional electrophoretic separation of oligonucleotides after complete T_1 RNAase digestion of fractions obtained from cells infected with ϕ80d*su*$^+$7(UAG) phage.

(a) tRNA originating from the high-mobility region in the 4 S band of a polyacrylamide gel. (b) tRNA purified first by aminoacylation with a high level of GlnRS, then phenoxyacetylation (Gillam *et al.*, 1968) and fractionation on a benzoylated DEAE-cellulose column with a salt–ethanol gradient. The spots numbered 1 to 14 are those that correspond to RNAase T_1 oligonucleotides obtained by analogous digestion of pure $tRNA^{Trp}$ (Hirsh, 1971). Spot 13, the major product, contained isopentenyl A(i^6A) whereas spot 13a, the minor product, is the fully modified oligonucleotide (ms^2i^6A). The anticodon containing oligonucleotide 13 migrates at pH 3·5, faster than its homologue from the *su*$^-$7 tRNA (see Plate II).

Plate II. Autoradiogram of a combined electrophoretic and homochromatographic separation of the complete RNAase T_1 digestion products of *su*$^+$7(UAG), *su*$^-$7 and *su*$^+$7(UAA/G) tRNA.

A complete RNAase T_1 digest of ^{32}P-labeled tRNA was fractionated in the first dimension by electrophoresis on cellulose acetate strips at pH 3·5 and 7 M-urea, and then the oligonucleotides were transferred to a DEAE-cellulose thin-layer chromatography plate and chromatographed at 60°C with homomixture C according to Brownlee & Sanger (1969). The faster migration of oligonucleotide 13 in the first dimension is what is expected with a C to U change between the *su*$^-$7 and the *su*$^+$7(UAG) tRNAs and with a change of 2 Cs to 2 Us between the *su*$^-$7 and the *su*$^+$7(UAA/G) tRNAs. The sequences of these anticodon-containing oligonucleotides were deduced as described in the legend to Table 5.

[*facing p. 254*

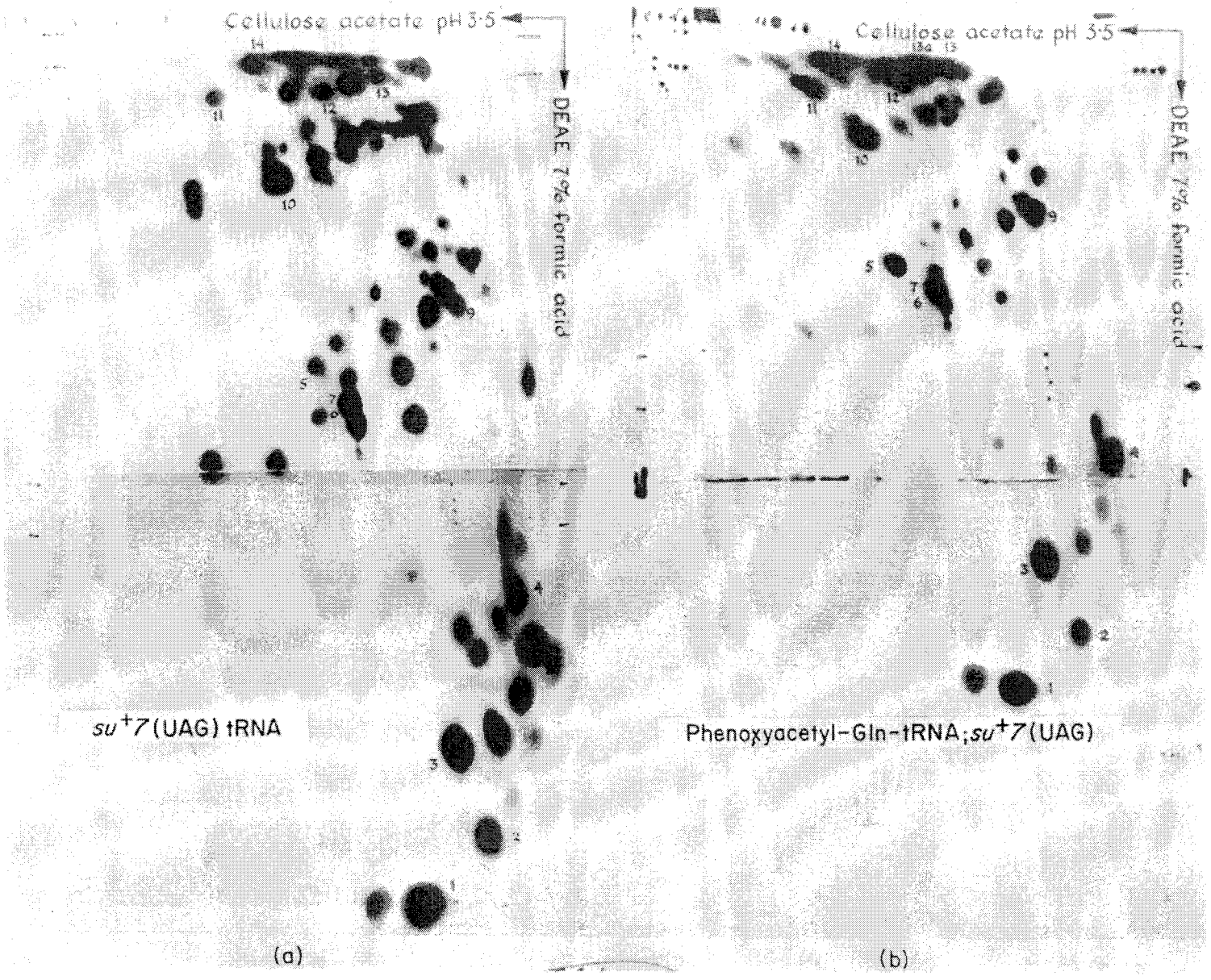
Cellulose acetate pH 3·5
DEAE 7% formic acid
su+7(UAG) tRNA
(a)
Cellulose acetate pH 3·5
DEAE 7% formic acid
Phenoxyacetyl-Gln-tRNA;su+7(UAG)
(b)

PLATE I.

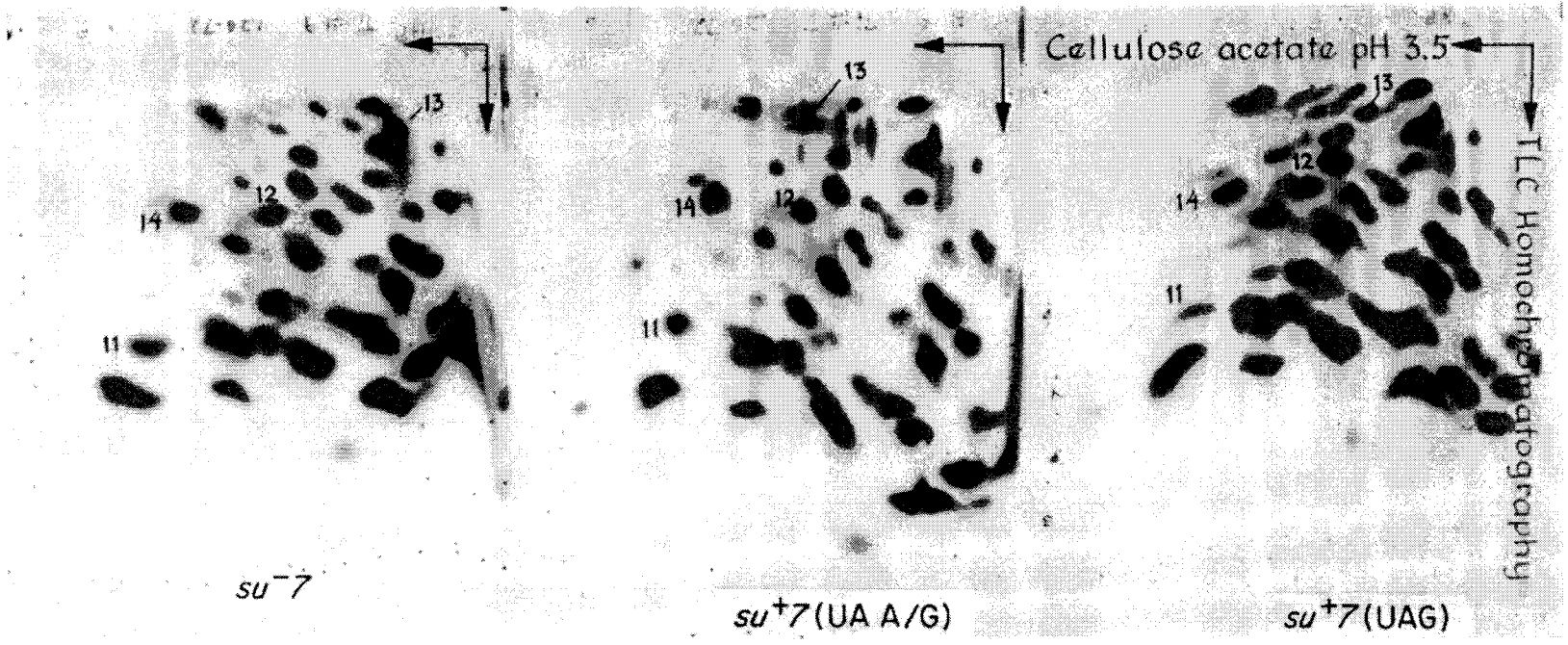
Cellulose acetate pH 3.5
TLC Homochromatography
13
12
14
11
su⁻7
su⁺7(UA A/G)
su⁺7(UAG)

PLATE II.

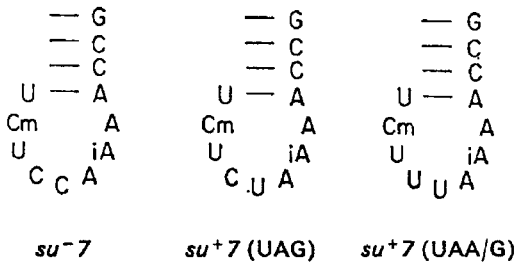

FIG. 2. Anticodon sequences of *su*$^-$*7*, *su*$^+$*7*(UAG) and *su*$^+$*7*(UAA/G) tRNAs.
The sequences were established as described in the legend to Table 5. The *su*$^-$*7* sequence is identical to the sequence of tRNA^{Trp} established by Hirsh (1971).

which normally translates UGG, can be altered to yield the *su*$^+$*7* tRNA, which translates UAG.

To establish that it is a glutamine-accepting tRNA in the *su*$^+$*7*(UAG) tRNA that generates the oligonucleotides characteristic of tRNA^{Trp}, the ^{32}P-labeled tRNA obtained from the acrylamide gel (see above) was esterified with glutamine using high levels of GlnRS, then phenoxyacetylated and chromatographed on benzoylated DEAE-cellulose (Gillam *et al.*, 1968). The RNAase T_1 fingerprint of this material (at least 50% pure as deduced from the ratio of the counts per phosphate in the pA-G spot relative to the T-Ψ-C-G spot) contained all the oligonucleotides characteristic of wild-type tRNA^{Trp} except for the oligonucleotide 13 containing the anticodon sequence (Plate I(b)). This oligonucleotide migrates at pH 3·5 faster than its homologue from the *su*$^-$*7* tRNA.

The tRNAs induced by infection with the phage containing the wild-type allele (*φ*80d*su*$^-$*7*) as well as with the ochre-suppressor phage *φ*80d*su*$^+$*7*(UAA/G) (derived from *φ*80d*su*$^+$*7*(UAG); Soll, 1974) were also examined. The complete RNAase T_1 digests of the ^{32}P-labeled tRNA were fractionated either by two-dimensional electrophoresis, or by homochromatography on cellulose–DEAE-cellulose thin-layer plates (Brownlee & Sanger, 1969). Once again, the tRNA^{Trp}-specific oligonucleotides were very prominent (Plate II). The only significant difference between the autoradiograms of the separated oligonucleotides from the three experiments is the position of spot 13. In each case, substitution of C by U as occurs in the change from *su*$^-$*7* to *su*$^+$*7*(UAG) and from *su*$^+$*7*(UAG) to *su*$^+$*7*(UAA/G) causes the oligonucleotide which forms spot 13 to migrate more rapidly in the first dimension. Since the chain length of the oligonucleotide is not altered, it is not surprising that mobility of each oligonucleotide in the second dimension of homochromatography remains unchanged (Brownlee *et al.*, 1968). The analyses of enzymatic digests of these oligonucleotides are presented in Table 5. The sequence of the oligonucleotide obtained from the tRNA produced after infection with the *su*$^-$*7* phage can be deduced because the genetic evidence (Soll, 1974) suggests that only a single base change converts *su*$^+$*7*(UAG) to *su*$^-$*7* (Soll, 1974); as expected, the sequence in *su*$^-$*7* is identical to that described by Hirsh (1971).

The sequence analysis of the oligonucleotide in spot 13 obtained after infection with *φ*80d*su*$^+$*7*(UAA/G) indicates that a second C to U transition in the anticodon

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sequence as described for tRNA^{Met} (Cory & Marcker, 1970) or as we observed for tRNA^{Gln} (Folk & Yaniv, unpublished observation). Another variation was that the oligonucleotide containing the anticodon contained both i^{6}A and ms^{2}i^{6}A, very likely also attributable to undermodification.

TABLE 5

Nucleotide sequence analysis of the oligonucleotides containing the anticodon sequences from $su^+7(UAG)$, su^-7 *and* $su^+7(UAA/G)$ *tRNAs*

Spot 13	Products produced by digestion with: RNAase U_2	Pancreatic RNAase	Pancreatic RNAase after carbodiimide modification	Deduced sequence
su$^+$*7*(UAG)	(C_m,U,U,U,C)A C-C-G A-A A	A-A-A-A-C C_m-U 2U 2C G	U*A-A-A-A-C U*C_m-U*C G* C U*C_m-U (minor)	U-C_m-U-C-U-A-A-A-A-C-C-G
su$^-$*7*	(C_m,U,U,C,C)A C-C-G A-A A	A-A-A-A-C C_m-U U 3C G	A-A-A-A-C U*C_m-U*C C G*	(U,C_m,U,C), C, A-A-A-A-C-C-G
su$^+$*7*(UAA/G)	(C_m,U,U,U,U,)A C-C-G A-A A	A-A-A-A-C C_m-U 3U C G	Not done	(U,U,U,C_m,U), A-A-A-A-C-G

RNAase T_1 oligonucleotides of spot 13, which contains the anticodon sequences, were isolated from a thin-layer chromatography plate after homochromatography for further RNAase U_2 digestion. Oligonucleotides eluted from DEAE-paper after electrophoresis were used for pancreatic RNAase digestion before and after soluble carbodiimide treatment (Cory & Marcker, 1970). (*) indicates bases modified with the carbodiimide. Conditions for digestion and fractionation of the products are described in Materials and Methods. The sequence of oligonucleotide 13 originating from the *su*$^+$7(UAG) tRNA could be unequivocally deduced from the digestion products. The digestion products of the *su*$^-$*7* and the *su*$^+$7(UAA/G) oligonucleotides agree with the expected U to C and C to U changes relative to the *su*$^+$*7*(UAG) sequence, respectively; their final sequences can be assigned as shown in Fig. 2. The sequence for the *su7* oligonucleotide is identical to the published sequence (Hirsh, 1971). The position of the isopentenyl A in the sequence A-A-A-A-C is assigned from Hirsh's published data.

is the basis of the new coding specificity of the *su*$^+$*7*(UAA/G) suppressor. Because the frequency with which this suppressor arises is so high we assume that it is related to *su*$^+$*7*(UAG) by a single base change (Soll, 1974) and, therefore, the sequence of this oligonucleotide can also be assigned.

4. Discussion

Our present finding that the suppressor mutation, *su*$^+$*7*(UAG), causes a base change in the anticodon of a tRNA, thereby enabling it to translate the termination signal UAG, is not unexpected. Nor is it surprising that the mutation affects a tRNA^{Trp} since that species' codon and UAG differ by only one base. Because there is only one structural gene for tRNA^{Trp} (Hirsh, 1971), we might reasonably have anticipated that this kind of suppressor mutation would have been lethal. What obscured this possibility from the beginning was the finding (Soll & Berg, 1969*b*) that glutamine and not tryptophan was incorporated in response to the UAG codon; and there was also the more plausible likelihood that the suppressor tRNA was derived from the CAG-specific tRNA^{Gln} (Folk & Yaniv, 1972). Now it is apparent that the single base change in the anticodon of tRNA^{Trp} alters not only the translational specificity of that tRNA from UGG to UAG, but also its amino acid-acceptor specificity from tryptophan to glutamine.

The acquisition of a new charging specificity and the loss of the old† poses some interesting questions about the basis of the selectivity of GlnRS and TrpRS for their cognate tRNAs. Other nonsense (Smith *et al.*, 1966; Hayashi & Söll, 1971) and missense suppressor mutations affecting anticodon sequences (see Berg, 1972) do not alter the specificity of charging although, in the latter instance, the affinities between some of the altered tRNA species and their cognate enzymes are markedly altered (Carbon & Curry, 1968; Squires & Carbon, 1971). TrpRS is unique, therefore, in that it fails to charge a cognate tRNA with but a single base change in the anticodon triplet; moreover, this is the first instance in which a single base change in the anticodon causes an aminoacyl-tRNA synthetase to mischarge a tRNA *in vivo*. Since the efficiency of suppression by *su*$^+$*7*(UAG) *in vivo* is as high as 75% (Soll & Berg, 1969*a*), it may be that the low efficiency of aminoacylation by glutamine seen *in vitro* is misleading and does not reflect the rate of the reaction *in vivo*.

Additional instances of mischarging of tRNA have come to light. Certain mutational changes in the *su*$^+$*3* suppressor species of tRNA^{Tyr} cause the insertion of an amino acid other than tyrosine (Shimura *et al.*, 1972; Hooper *et al.*, 1972). More recently Smith & Celis (1973) and Celis *et al.* (1973) have reported that single base changes in the C-C-A stem of the UAG-suppressor tRNA^{Tyr} species can convert that tRNA to a glutamine-inserting suppressor *in vivo* and to a glutamine acceptor *in vitro*. With certain, but not all, of the base changes in this region a loss of tyrosine-acceptor activity accompanies the acquisition of glutamine-acceptor specificity.

Yarus (1972*a*,*b*) and his colleagues (Mertes *et al.*, 1972) have noted a surprising degree of mischarging of tRNAs *in vitro* by only slight modifications in the reaction conditions. One might wonder why enhancement of such "natural" mischarging reactions are not more frequently detected as suppressors. But clearly, unless translation by such mischarged tRNAs is restricted to nonsense or very infrequently

† The UAG-specific tRNA^{Gln} (peak 1 in Fig. 1) does not accept tryptophan even with purified TrpRS (unpublished observations).

used codons, the consequence of such mischarging is likely to be deleterious to the amino acid sequence fidelity of proteins.

Without knowing more about the structural basis for the recognition between GlnRS and its cognate tRNAs, one can only guess what causes the enzyme to recognize, albeit poorly, the modified $tRNA^{Trp}$ and the altered forms of $tRNA^{Tyr}$. A comparison of the nucleotide sequences of $tRNA^{Trp}$ and one of the $tRNA^{Gln}$ species (Fig. 3) reveals few similarities except for the anticodon and C-C-A stems. The

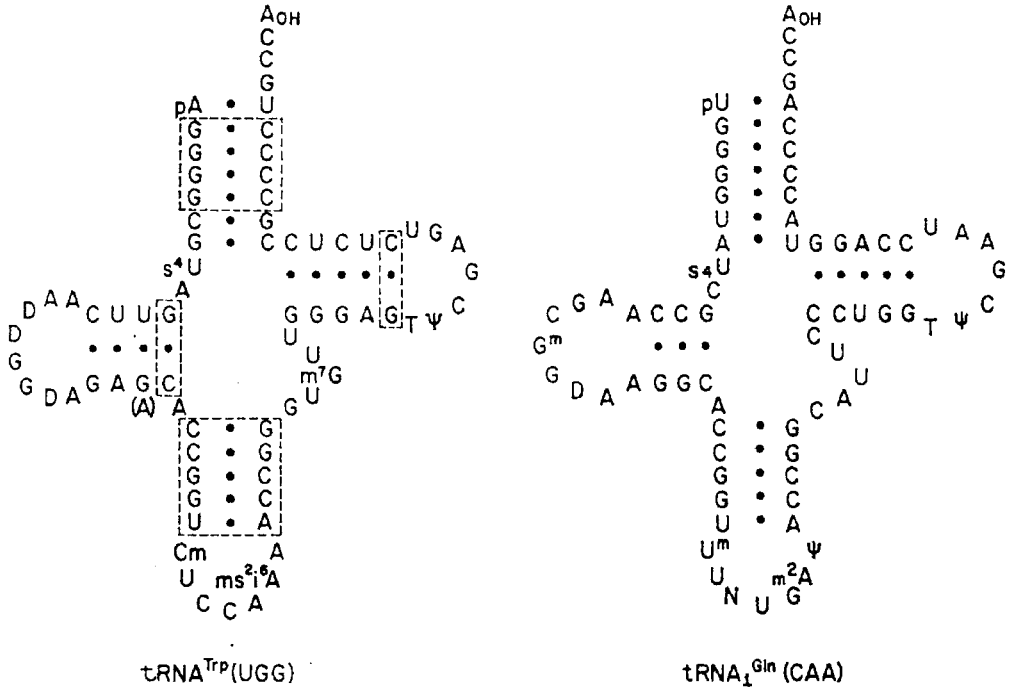

FIG. 3. Nucleotide sequences of $tRNA^{Trp}$ and $tRNA_1^{Gln}$. The base-pairs common to both sequences are enclosed in boxes.

base changes that convert the *su*$^+$*3* $tRNA^{Tyr}$ species to a glutamine acceptor are also not easily interpretable in terms of a recognition sequence within the affected regions. Perhaps further studies with a variety of base changes will be able to discern the role these regions have in the reaction with GlnRS. Especially useful in such a study would be a determination of the activity and specificity with which other mutationally altered forms of the $tRNA^{Trp}$ species react with GlnRS, TrpRS and possibly with other enzymes; i.e. what effect do the substitutions in the anticodon sequence, which permit $tRNA^{Trp}$ to translate UAA/G or UGA (Soll, 1974), have on the amino acid acceptor specificity of the tRNA?

The mutational change *su*$^-$*7* to *su*$^+$*7*(UAG) is lethal to the cell that harbors only one *su7* locus. The loss of capacity to translate UGG as tryptophan during protein synthesis makes the lethality not surprising. Quite clearly in *su*$^+$*7*/*su*$^-$*7* heterozygotes, the suppressor activity is dominant while the lethality is recessive. It seems reasonable to assume that the UAG and UGA recessive lethal suppressors found by Miller & Roth (1971) in *S. typhimurium* are analogous to those we have described here, i.e. they are due to mutationally altered $tRNA^{Trp}$ species with the appropriate anticodon changes. However, the recessive lethality of the UGA

suppressor is surprising since the base change in the "third" base of the anticodon should, because of "Wobble" (Crick, 1966), allow that tRNA to translate both UGA and UGG; conceivably, that base change also alters its ability to accept tryptophan efficiently. Consistent with this notion is the observation that tryptophan-inserting UGA suppressors isolated in haploid *E. coli* do not contain alterations in the *anticodon* of the $tRNA^{Trp}$; rather, they contain substitutions outside the anticodon that modify the coding properties of those tRNAs (Hirsh, 1971).

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